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14. ABSTRACT

Hypoxia is a potent microenvironmental factor promoting metastatic progression. A critical step in metastatic tumor progression is the ability of tumor cells to evade immune attack. Tumor cells utilize a complex set of mechanisms that prevent the immune system from mounting effective anti-tumor responses. Moreover, the hypoxic tumor microenvironment plays an important role in immune escape by favoring immune suppression and tumor resistance. Tumor hypoxia is thought to promote the immunosuppressive phenotypes of both tumor cells as well as infiltrating immune cells. However, the mechanisms by which hypoxia promotes immunosuppression in ovarian cancer remains to be elucidated and may have important therapeutic implications in the treatment of metastatic ovarian cancer. We hypothesize that hypoxia through HIF-1 signaling in regulatory T cells promotes angiogenic and immunosuppressive phenotypes, each contributing to metastatic ovarian cancer tumor growth. Here we generated mice to directly assess the functional role of HIF-1 in Treg cells in ovarian cancer metastatic tumor growth, angiogenesis, and immunosuppression.

15. SUBJECT TERMS
Ovarian cancer, hypoxia, tumor microenvironment, immune suppression, angiogenesis

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INTRODUCTION:

Metastatic disease is the leading cause of death in ovarian cancer patients. Metastasis is a highly complex and dynamic process that involves critical interactions between tumor cells and the microenvironment. Hypoxia is a potent microenvironmental factor promoting metastatic progression. Clinically, hypoxia and the expression of the hypoxia inducible transcription factors HIF-1, and HIF-2 are associated with increased distant metastasis and poor survival in ovarian cancer (1). A critical step in metastatic tumor progression is the ability of tumor cells to evade immune attack. Tumor cells utilize a complex set of mechanisms that prevent the immune system from mounting effective anti-tumor responses. Moreover, the hypoxic tumor microenvironment plays an important role in immune escape by favoring immune suppression and tumor resistance. Tumor hypoxia is thought to promote the immunosuppressive phenotypes of both tumor cells as well as infiltrating immune cells (2). However, the mechanisms by which hypoxia promotes immunosuppression in ovarian cancer remains to be elucidated and may have important therapeutic implications in the treatment of metastatic ovarian cancer. Regulatory T cells (Tregs) are an important component of the immunosuppressive tumor microenvironment in ovarian cancer. Recent studies have suggested that hypoxic ovarian cancer cells promote the recruitment of Tregs, which in turn promotes immune tolerance and angiogenesis (3). However, the role of the hypoxic tumor microenvironment in controlling Treg function remains unknown. We hypothesize that hypoxia and the activation of hypoxic signaling mediated by the hypoxic inducible transcription factor HIF-1 in Tregs promotes angiogenic and immunosuppressive phenotypes, each contributing to metastatic ovarian cancer tumor growth. Here we will determine the functional role of HIF-1 in Treg cells by utilizing a genetic approach to dissect the functions of HIF in the context of ovarian cancer metastatic tumor growth, angiogenesis, and immunosuppression.

KEYWORDS: Hypoxia, tumor microenvironment, ovarian cancer, regulatory T cell, HIF-1, angiogenesis, therapy, metastasis, immune suppression.

ACCOMPLISHMENTS:

The major goals of this project are to determine the functional role of hypoxic HIF signaling in regulatory T cells and the impact on ovarian cancer metastasis. In aim 1 we proposed to determine the role of HIF-1 deletion in Treg cells in ovarian tumor metastasis (100% completed in years 1 and 2). We generated mice to conditionally inactivate HIF-1 in Treg cells and evaluated the effect on metastatic ovarian cancer growth. In our last report, we demonstrated that 1) **HIF-1 inactivation in FOXP3 regulatory T cells (Tregs) does not affect the frequency of Tregs in the spleen, mesenteric lymph node, ascites, or tumor of ID8 tumor bearing mice (Fig. 1) and 2) metastatic tumor burden in the ID8-ascites model of ovarian cancer was comparable in the FOXP3-Cre and FOXP3-HIF1 deficient mice (Fig. 2).** In the second aim, we proposed to determine the role of HIF-1 deletion in regulating proangiogenic activities of Treg cells (60% complete). In the third aim, we will test the role of HIF-1 in mediating the suppressive function of Treg cells. This project investigates the role of hypoxia inducible factors in driving the metastatic phenotype of ovarian cancer and proposes to block these factors and associated pathways as therapeutic strategies for the treatment of ovarian cancer.

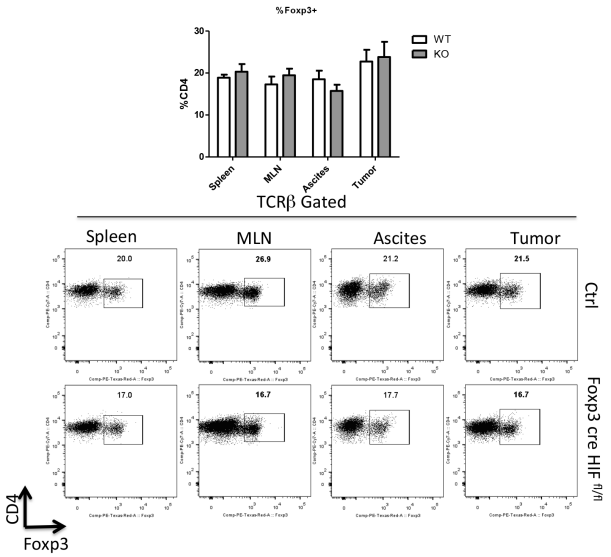

Figure 1. Frequency of Tregs in the spleen, mesenteric lymph node (MLN), ascites, or tumor of ID8 tumor bearing mice. FOXP3-Control (WT) or FOXP3-HIF-1 deficient mice were injected i.p. with 10^6 ID8-Ascites cells. The frequency of TCRB, CD4, and FOXP3 positive Tregs within each tissue was determined by FACS analysis 30 days after tumor inoculations.

The major goals of the project during this funding period are as stated in the approved SOW are as follows:

TASK 2. Determine the role of Treg HIF-1 on tumor angiogenesis (years 3 and 4.5).

Task 2a. *The role of Treg HIF-1 in regulating angiogenesis in ID8 tumors. (Months 24-30)*

To test the functional role of Treg HIF-1 in regulating ovarian cancer angiogenesis in vivo, ID8 tumor sections and ascites were analyzed from FOXP3-Cre control mice and FOXP3-HIF-1 mice described in Aim 1. VEGFA protein levels were measured in the ascites using the mouse VEGFA ELISA kit from R&D. Secreted VEGFA levels within the ascites fluid of FOXP3-Cre and FOXP3-HIF1 tumors were similar (Fig. 3A). Tumor sections were stained and quantified for CD31, an endothelial cell marker. The number of CD31 positive vessels per field were counted and were found be similar between FOXP3-Cre control and FOXP3-HIF1 deficient mice (Fig. 3B-C). Collectively, our data demonstrate that HIF-1 signaling in Tregs is not required for peritoneal tumor growth, ascites development or angiogenesis within ID8 tumors. These data suggest that other cell types and/or signaling pathways contribute peritoneal tumor vascularization and growth within the peritoneal cavity.

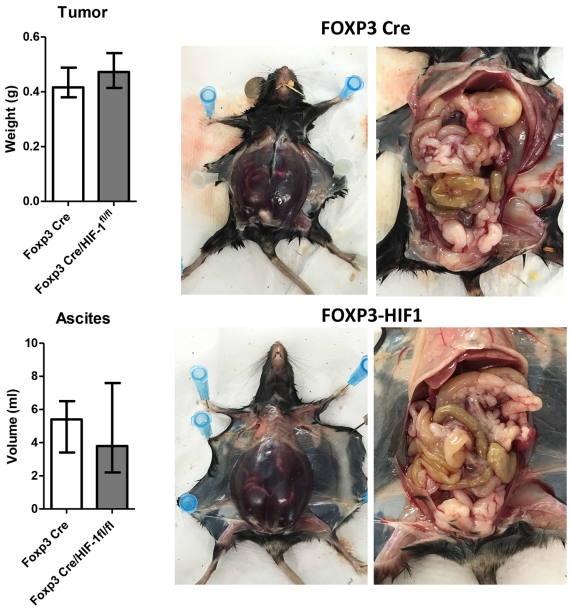

Figure 2. Metastatic tumor burden in the ID8 model of ovarian cancer. FOXP3-Cre control and FOXP3-HIF1 deficient female mice were injected i.p. with $1X10^6$ ID8-Ascites cells. At 30 days of injection mice developed symptoms of ovarian cancer. Ascites volume was measured using a syringe. Macroscopic tumors in the peritoneum were collected and weighed (n=8).

Task 2b. *The role of Treg HIF-1 in regulating angiogenesis in vivo. (Months 30-36)*

To directly assess the role of Treg HIF-1 in regulating angiogenesis in vivo, we proposed to determine the number of CD31+ endothelial cells in subcutaneous matrigel plugs that contain conditioned media from normoxic or hypoxic (2% oxygen) CD4+CD25+ T cells isolated from FOXP3-Cre control or FOXP3-HIF-1 deficient mice after 72 hours of incubation. This protocol requires isolation of CD4+CD25+ regulatory T cells from the YFP expressing FOXP3-Cre control and FOXP3-HIF-1 deficient mice using the Aria cell sorter, culturing and incubating the cells under normoxic or hypoxic conditions (72 hours) to collect conditioned media. The conditioned media containing the proangiogenic factors is then mixed with matrigel and tested for in vivo angiongenic potential when incubated in the subcutaneous space of mice for 72 hours.

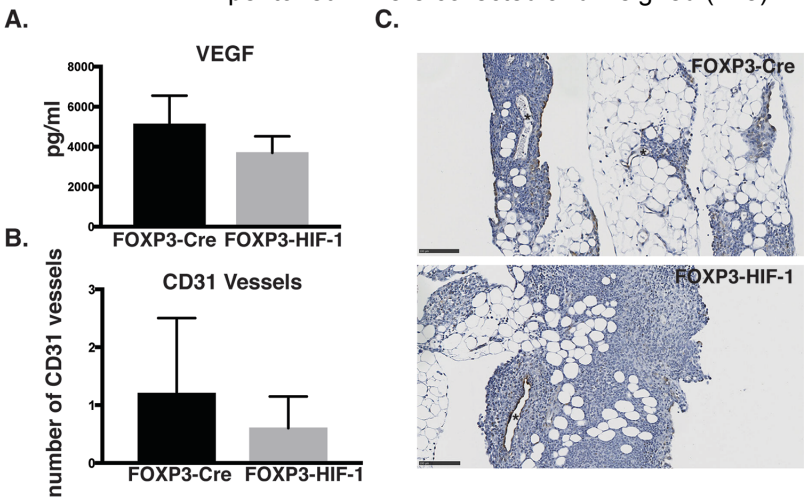

Figure 3. Inactivation of HIF-1 in Tregs does not affect ovarian tumor vascularization. FOXP3-Cre control and FOXP3-HIF1 deficient female mice were injected i.p. with $1X10^6$ ID8-Ascites cells. At 30 days of injection mice developed symptoms of ovarian cancer. **A.** VEGF protein levels were measured in ascites fluid using an ELISA kit. **B.** Blood vessels lined by CD31 were stained by immunohistochemistry for CD31 and the numbers of CD31 lined vessels in a 100um filed were counted (left). Representative pictures on right (n=6).

We followed standard protocols for regulatory T cell isolation and culture *in vitro*. We were able to obtain 99% pure Treg cultures that were viable under normoxic conditions. However, we routinely observed that hypoxia resulted in at least a 2-fold reduction in Treg number in FOXP3-Cre cultures compared to Tregs cultured under normoxic conditions (Fig 4A). This result suggests that hypoxia inhibits the proliferation, survival and/or phenotype of regulatory T cells. Importantly, the reduction in Treg cell number under hypoxic conditions will influence the results in our assay where we test the angiogenic potential of conditioned media from normoxic and hypoxic FOXP3-Cre and FOXP3-HIF-1 Tregs. For example, if we observe reduced angiogenic potential of conditioned media collected from hypoxic Tregs compared to normoxic Tregs we will not know if this is this because hypoxia reduces the angiogenic potential of the cells or we simply have fewer cells secreting proangiogenic factors. This result was unexpected given that previous studies indicated that hypoxia promotes Treg induction and recruitment (3, 4). Recent reports demonstrating that hypoxia reduces the proliferation and survival of regulatory T cells in vitro support our data (4, 5). Lee et al. further demonstrated that HIF signaling in Tregs converts them from Tregs into Th1-effector T cells (5). Taking our results together with the results from Lee et al. lead us to hypothesize that hypoxia and HIF-1 signaling in committed Tregs within the tumor microenvironment reduces their abundance. To begin to test this hypothesis, we examined if Tregs are abundant within hypoxic zones of the ovarian cancer microenvironment. Tumor sections from the ID8 tumor bearing mice in Aim 1 were stained for Tregs (FOXP3) and hypoxia (pimonidazole, PIMO). Strikingly, we found that Tregs are excluded from hypoxic zones within the ovarian tumor microenvironment (Fig. 4B). This finding is consistent with our data demonstrating no significant change in vascularization or ID8 tumor burden in FOXP3-HIF1 deficient mice compared to FOXP3-Cre control mice. Overall, our data suggest that in the tumor microenvironment, Tregs are not localized to hypoxic regions and HIF deletion in Tregs does not have a significant change in vascularization or tumor burden. Based on our data and current data in the literature we now hypothesize that there may be differential roles for hypoxia and HIF signaling in Treg induction versus Treg maintenance.

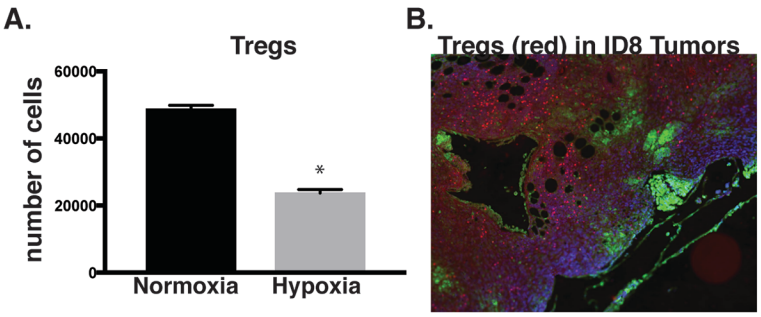

Figure 4. Hypoxia decreases Treg numbers. A. Purified Tregs from FOXP3-Cre mice were purified using FACS sorting. Tregs were cultured under normoxia (21%) or hypoxic (2%) oxygen conditions for 72 hours. The numbers of cells were counted. **B.** Immunofluorescent staining of FOXP3 positive Tregs (red) and hypoxia (Pimonidazole, green) in ID8 ovarian omental metastases. Blue shows DAPI positive nuclei.

What opportunities for training and professional development has the project provided?

This grant is a career development grant where I am an active member and participant of the Ovarian Cancer Academy. During this funding period (July 31, 2017- July 31, 2018) I attended the DOD Ovarian Cancer Academy (DOD OCA) meeting in Pittsburgh (October, 2017) where I had the opportunity to network and meet with the Deans of the Academy, Drs. Nita Maihle and Doug Levine, as well as all of the other early career investigators within the Ovarian Cancer Academy. Importantly, I also had the opportunity to get to talk with many of the patient advocates that attended this meeting and greatly enjoyed this experience. It helped me to gain an better understanding of their experience receiving therapy and the types of new therapies that they would be looking for. I also attend and participate in our monthly DOD OCA webinars where I have had the opportunity to present my work and receive feedback, learn about others work to identify collaborations, and receive career development lectures. The career development lecture of drug development and obtaining funding for the development of new agents was very helpful. Finally, I have had the opportunity to attend the AACR ovarian cancer meeting (October 2017). Additional professional development activities include organizing and hosting an Ovarian Cancer Focus Group meeting at Stanford University where Ovarian cancer researchers (Oliver Dorigo, Jonathan Berek, Mickey Hu, Nelson Teng, and Wendy Fantl) present their work in an informal setting to establish collaborations and receive constructive feedback for their work. For my training

activities, I meet with my mentor, Dr. Jonathan Berek, monthly to discuss the progress and growth of my ovarian cancer research and identify opportunities for growth. As a result of these meetings, I have applied and received additional extramural funding from the Marsha Rivkin Center for Ovarian Cancer Research, the Mary Kay Foundation and the Department of Defense OCRP to support my ovarian cancer research.

How were the results disseminated to communities of interest?
I have reached out to the greater Stanford community to make them aware of my project activities and involvement with the DoD Ovarian Cancer Academy. I was interviewed by the Stanford Medicine Scope Blog, an online publication for the Stanford Community and donors, where I described the need for ovarian cancer research, the goals of the DoD Ovarian Cancer Academy, as well as my professional and research goals within this program. I have also presented an invited oral presentation on my work on Hypoxia and Ovarian cancer supported by this grant at 1) the Keystone Symposia in Whistler, British Columbia, Canada in March 2017 and 2) the Tumor Microenvironment Workshop in Miami, FL in May 2017 and 3) a poster at the AACR Ovarian Cancer meeting last October in Pittsburgh in October 2017. I also have shared my work in ovarian cancer with Sue McCollum, a breast cancer survivor and founder of My Blue Dots, whom has provided updates about my work and announced my funding from the DoD OCA on her monthly newsletter and website.

What do you plan to do during the next reporting period to accomplish the goals?
Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.
The proposed goal of the research in the next reporting period is to:

Task 2b. *The role of Treg HIF-1 in the production of VEGFA in vitro. (Months 36-42)*
To determine the role of Treg HIF-1 in the production of VEGFA, YFP sorted Treg cells from FOXP3-Cre and FOXP3-HIF-1 mice will be cultured under normoxic or hypoxic (2% oxygen) conditions for 48 hours. Secreted VEGFA levels in the conditioned media will be compared between all groups using a mouse VEGFA ELISA kit as previously described (Rankin et al., 2012).

TASK 3. Determine the role of Treg HIF-1 on the immunosuppressive phenotype (years 4.5 and 5).
Task 3a. *The role of Treg HIF-1 in suppressive function in vitro. (Months 42-48)*
Previous studies have demonstrated that HIF-1 deficient Tregs using Lck-Cre have a significant defect in suppressing the proliferation of CD4 T cells (Clambey et al., 2012). We will compare the suppressive function of FOXP3-Cre Tregs and FOXP3-HIF-1 deficient Tregs in vitro under normoxic and hypoxic conditions. YFP CD4+ CD25+ Treg cells isolated from lymph node will be sorted and subjected to an in vitro CD4 T cell proliferation assay. This assay will be performed in collaboration with Dr. Edgar Engleman's lab (Fernandez et al., 2007). In summary, CD4+ T cells and CD4+ CD25+ Tregs will be isolated from the lymph node and stimulated with anti-CD3 antibody along with beads coated with antibody to stimulate proliferation. The Treg to CD4 T cell ratios will be established and CFSE labeled CD4 T proliferation will be measured by flow cytometry.

**Since the proposed in vitro assays require culturing Tregs under hypoxic conditions, where we have now observed a significant reduction in cell viability/growth, I am contacting my Grant Officer to determine if it is possible to revise these aims as I now believe that we cannot properly interpret the data from these studies.

IMPACT:
What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report.

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project.
Nothing to Report.

What was the impact on other disciplines?
Nothing to Report.

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.
What was the impact on technology transfer?
Nothing to Report.

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including: transfer of results to entities in government or industry; instances where the research has led to the initiation of a start-up company; or adoption of new practices. What was the impact on society beyond science and technology?
Nothing to Report.

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
Nothing to Report.

CHANGES/PROBLEMS:
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.
Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.
Nothing to report.

PRODUCTS: (during the reporting period)
Publications
Nothing to Report

Books
Nothing to Report

Presentations

2017 Hypoxic signaling in Tumor-Mesothelial Niche Promotes Collagen Remodeling and Ovarian Cancer Metastasis. 15th International Tumor Microenvironment Workshop, Miami, FL

2017 Hypoxic signaling in the tumor-mesothelial niche. Keystone Symposia, Whistler, Canada

2017 Hypoxic signaling in the tumor-mesothelial niche promotes collagen remodeling and ovarian cancer metastasis. AACR: Ovarian Cancer Meeting, Pittsburgh, PA

Websites
Nothing to Report

Technologies or techniques
Nothing to Report

Inventions, patents, licenses
Nothing to Report

Other Products
We have generated FOXP3-HIF-1 mice in which HIF-1 is conditionally inactivated in regulatory T cells (Tregs). These mice can be useful for a variety of applications investigating the impact of HIF-1 signaling in Treg function.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Erinn Rankin
Project Role:	Primary Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Dr. Rankin has designed and assisted Ms. Foreman in all proposed experimental design and execution.
Funding Support:	

Name:	Jonathan Berek
Project Role:	Mentor
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.2
Contribution to Project:	Dr. Berek mentors Dr. Rankin by ensuring that Dr. Rankin's research and career development is progression.
Funding Support:	N/A

Name:	Yiren Xiao
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3.85
Contribution to Project:	Dr. Xiao has performed proposed experiments with Dr. Rankin.
Funding Support:	N/A

Name:	Suchitra Natarajan
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3.78
Contribution to Project:	Dr. Natarajan has performed proposed experiments with Dr. Rankin.
Funding Support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
There has not been a change in active Other Support for my PD/PIs or senior/key personnel. However, my research assistant Katie Foremen left for medical school in June 2017. Suchitra Natarajan, postdoctoral fellow joined the project at this time (June 2017). Yiren Xiao, postdoctoral fellow also contributes to this project.

What other organizations were involved as partners?
Nothing to Report.

SPECIAL REPORTING REQUIREMENTS
Nothing to Report.

APPENDICES
References
Erinn B. Rankin cv

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Educational Background

2002-2007	University of Pennsylvania, Philadelphia, PA Cell Growth and Cancer (Dr. Volker Haase) The role of hypoxia inducible factors family members in the development of VHL disease. January 1, 2007. Dissertation available from ProQuest. Paper AAI3260971.	Ph.D.
1996-2000	University of Illinois Urbana-Champaign, IL Microbiology	B.S

Professional Appointments

2014–present	Assistant Professor Department of Radiation Oncology, Department of Obstetrics and Gynecology, Stanford University, Stanford, CA
2012–2014	Research Associate Department of Radiation Oncology, Stanford University, Stanford, CA
2010–2011	Visiting Research Scholar (Dr. Ernestina Schipani) Endocrine Unit, Massachusetts General Hospital, Boston, MA
2007–2012	Postdoctoral Scholar (Dr. Amato J. Giaccia) Department of Radiation Oncology, Stanford University, Stanford, CA

Other Professional Positions

2000-2002	Research Specialist (Dr. EunRan Suh) University of Pennsylvania, Philadelphia, PA

Honors and Awards

2016	Mary Kay Foundation Research Award
2016	Pape Family Pilot Award, Rivkin Center for Ovarian Cancer Research
2015	Ovarian Cancer Academy Award, Department of Defense
2014-2016	Gabilan Faculty Award, Stanford University
2012	J. Martin Brown Award for Outstanding Achievements in the Radiation Sciences, Stanford University
2011	Travel Award, Keystone Symposia
2007-2012	Postdoctoral Trainee Award, NCI
2007	Saul Winegrad Award for Outstanding Dissertation, University of Pennsylvania
2005-2007	Pre-Doctoral Trainee Award, American Heart Association

Publications ***(Peer Reviewed Journal Articles)***

1. Wu PH, Onodera Y, Ichikawa Y, **Rankin EB**, Giaccia AJ, Watanabe Y, Qian W, Hashimoto T, Shirato H, Nam JM. Targeting integrins with RGD-conjugated gold nanoparticles in radiotherapy decreases the invasive activity of breast cancer cells. **Int J Nanomedicine.** 2017 Jul 14;12:5069-5085.
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16. **Rankin EB**, Fuh KC, Taylor TE, Krieg AJ, Musser M, Yuan J, Wei K, Kuo CJ, Longacre TA, Giaccia AJ. AXL is an essential factor and therapeutic target for metastatic ovarian cancer. **Cancer Res.** 2010 Oct 1;70(19):7570-9. doi: 10.1158/0008-5472.CAN-10-1267. Epub 2010 Sep 21. (PMID: 20858715).
17. Krieg AJ, **Rankin EB**, Chan D, Razorenova O, Fernandez S, Giaccia AJ. Regulation of the histone demethylase JMJD1A by hypoxia-inducible factor 1 alpha enhances hypoxic gene expression and tumor growth. **Mol Cell Biol.** 2010 Jan;30(1):344-53. doi: 10.1128/MCB.00444-09. (PMCID: PMC2798291)
18. **Rankin EB,** Rha J, SelakMA, Unger TL, Keith B, Liu Q and Haase VH. HIF-2 regulates hepatic lipid metabolism. **Mol Cell Biol.** 2009 Aug;29(16):4527-38. doi: 10.1128/MCB.00200-09. Epub 2009 Jun 15. (PMCID: PMC2725738)
19. **Rankin EB**, Rha J, Unger TL, Wu CH, Shutt HP, Johnson RS, Simon MC, Keith B, Haase VH. Hypoxia-inducible factor-2 regulates vascular tumorigenesis in mice. **Oncogene.** 2008 Sep11;27(40):5354-8. doi: 10.1038/onc.2008.160 Epub 2008 May 19. (PMCID: PMC2575082)
20. Peyssonnaux C, Zinkernagel AS, Schuepbach RA, **Rankin E**, Vaulont S, Haase VH, Nizet V, Johnson RS. Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). **J Clin Invest.** 2007 Jul;117(7):1926-32. (PMCID: PMC1884690)
21. **Rankin EB,** Biju MP, Liu Q, Rha J, Johnson RS, Simon CM, Keith B, Haase VH. Hypoxia inducible factor (HIF)-2 regulates hepatic EPO expression in vivo. **J Clin Invest.** 2007 Apr;117(4):1068-77. (PMCID: PMC1838939)
22. **Rankin EB,** Tomaszewski JE, Haase VH. Renal cyst development in mice with conditional inactivation of the von Hippel-Lindau tumor suppressor. **Cancer Res.** 2006 Mar;66(5):2576-2583. (PMCID: PMC3514875)
23. **Rankin EB**, Higgins DF, Walisser JA, Johnson RS, Bradfield CA, Haase VH. Inactivation of the arylhydrocarbon receptor nuclear translocator (Arnt) suppresses von Hippel-Lindau disease associated vascular tumors in mice. **Mol Cell Biol.** 2005 Apr;25(8):3163-72. (PMCID: PMC1069599)
24. **Rankin EB**, Xu W, Silberg DG, Suh E. Putative intestine-specific enhancers located in 5' sequence of the CDX1 gene regulate CDX1 expression in the intestine. **Am J Physiol Gastrointest Liver Physiol.** 2004 May;286(5):G872-80. doi: 10.1152/ajpgi.00326.2003. Epub 2004 Jan 8. (PMID: 14715525)
25. **Rankin EB**, Yu D, Jiang J, Shen H, Pearce EJ, Goldschmidt MH, Levy DE, Golovkina TV, Hunter CA, Thomas-Tikhonenko A. An essential role of Th1 responses and interferon gamma in infection-mediated suppression of neoplastic growth. **Cancer Biol Ther**. 2003 Nov-Dec;2(6):687-93. (PMID: 14688478)
26. Suh ER, Ha CS, **Rankin EB**, Toyota M, Traber PG. DNA methylation down regulates CDX1 gene expression in colorectal cancer cell lines. **J Biol Chem.** 2002 Sep 27;277(39):35795-800. doi: 10.1074/jbc.M205567200. (PMID: 1212493)

Submitted

Natarajan S, Foreman K, Shehade H, Fregoso D, Soriano M, Eggold JT, Krishnan V, Heilshorn S, Dorigo O, Sinha S, Fuh K, **Rankin EB**. Hypoxic signaling in tumor-mesothelial niche promotes collagen remodeling and ovarian cancer metastasis.

Peer –reviewed Review Articles

1. Eggold JT and **Rankin EB**. Erythropoiesis, EPO, macrophages, and bone. **Bone**. 2018 Mar 15. pii: S8756-3282(18)30121-2.
2. **Rankin EB** and Giaccia AJ. The Receptor Tyrosine Kinase AXL in Cancer Progression. **Cancers (Basel)**. 2016 Nov 9;8(11).
3. **Rankin EB**, Nam JM, and Giaccia AJ. Hypoxia: Signaling in the metastatic cascade. **Trends in Cancer.** 2016 Jun 2 (6): 295–304.

4. **Rankin EB** and Giaccia AJ. Hypoxic control of metastasis. **Science.** 2016 Apr 8;352(6282):175-80.
5. **Rankin EB**, Narla A, Park JK, Lin S, Sakamoto KM. Biology of the bone marrow microenvironment and myelodysplastic syndromes. **Mol Genet Metab.** 2015 Sep-Oct; 116(1-2):24-8. doi: 10.1016/j.ymgme.2015.07.004. PMID: 26210353
6. Wu C, Giaccia AJ, and **Rankin EB**. Osteoblasts: A novel source of erythropoietin. **Curr Osteoporos Rep.** 2014 Dec;12(4):428-32. doi: 10.1007/s11914-014-0236-x. PMID: 25204993.
7. Schipani E, Wu C, **Rankin EB**, and Giaccia AJ. Regulation of bone marrow angiogenesis by osteoblasts during bone development and homeostasis. **Front Endocrinol** 2013 Jul 10;4:85
8. Wu C, **Rankin EB**, and Giaccia AJ. Blood and bones: Osteoblastic HIF signaling regulates erythropoiesis. **Cell Cycle** 2012 Jun 15;11(12)2221-2.
9. **Rankin EB**, Giaccia AJ, Schipani E. A central role for hypoxia signaling in cartilage, bone, and hematopoiesis. **Curr Osteoporosis Rep.** 2011 Jun;9(2):46-52.
10. **Rankin EB**, Giaccia AJ, Hammond EM. Bringing H2AX into the angiogenesis family. **Cancer Cell.** 2009 Jun 2;15(6):459-61.
11. **Rankin EB** and Giaccia AJ. The role of hypoxia-inducible factors in tumorigenesis. **Cell Death Differ.** 2008 Apr;15(4):678-85.

Book Chapters

1. **Rankin, EB**, Erler, J and Giaccia, AJ. The cellular microenvironment and metastasis. In: Abeloff's Clinical Oncology, 5th Edition. Niederhuber, J.E., Armitage, J.O., Doroshow, J.H., Kastan, M.B. and Tepper, J. (eds), Elsevier 2014:40-51.
2. **Rankin, EB** and Giaccia, AJ. The cellular microenvironment and metastasis. In: Abeloff's Clinical Oncology, 6th Edition. Niederhuber, J.E., Armitage, J.O., Doroshow, J.H., Kastan, M.B. and Tepper, J. (eds), Elsevier *In press*
3. **Rankin, EB** and Sakamoto, KM. The Cellular and Molecular Mechanisms of Hematopoiesis. In: Pediatric Oncology, 1st Edition. Kupfer, G, Reaman, G.H., and Smith, F.O. (eds), Springer Nature *In press*

Editorial Service

Ad hoc Journal Reviewer

Cancer Discovery
Cell Reports
Journal of Clinical Investigation
Nature Communications
Oncogene

Grant Support

Current Funding

1. DoD OCRP Pilot Award (Rankin, P.I.) 09/30/17-09/29/19
   Preclinical Testing of FLASH Radiotherapy and Immune Checkpoint Blockade Combination Therapy in Ovarian Cancer
2. Mary Kay Foundation (Rankin, P.I.) 07/01/16-06/30/18
   Hypoxic Signaling in Metastasis: Molecular Mechanisms and Targeted Therapy
3. NCI RO1 (Giaccia, P.I.; Rankin, co-Investigator) 07/01/15-06/30/20
   Preclinical Testing of a Novel Therapy Targeting AXL in Advanced Kidney Cancer
4. DoD Ovarian Cancer Academy Award (Rankin, P.I.) 07/01/15-06/30/20
   The Role of Hypoxia in the Tumor Microenvironment: Implications for Ovarian Cancer Therapy

Pending Funding

1. NCI PO1 (Giaccia, P.I., Rankin Project 4 Lead) 04/01/19-03/31/24
   Tumor Hypoxia: Molecular Studies and Clinical Exploitation

2. ACS Research Scholar (Rankin, P.I.) 01/01/19-12/31/22
FTO in Kidney Cancer: Molecular Mechanisms and Targeted Therapy

Prior Funding

1. Marsha Rivkin Center for Ovarian Cancer Pilot Award (Rankin, P.I.) 04/01/16-03/31/17
"Targeting the hypoxic secretome in omental metastasis"
2. MD Anderson/KCRP (Pilot Award) (Giaccia, P.I.; Rankin, Co-Investigator) 04/01/13-03/31/14
"Mechanisms of tumor resistance to targeted RTK therapy in ccRCC"

Service as Grant Review

Grant Review Committees

Marsha Rivkin Center for Ovarian Cancer 2016-2017
Tina's Wish 2016,2018

Patents

Inhibition of AXL signaling in anti-metastatic therapy.
US Patent PCT/US2011/022125

Modified AXL peptides and their use in inhibition of AXL signaling in anti-metastatic therapy.
US Patent PCT/US2013/074786

University Administrative Service

Committee Service

PhD Thesis Committee

Department of Immunology, *PhD Thesis Committee Chair* 2018
Shelley Ackerman
Department of Radiation Oncology, *PhD Thesis Committee Chair* 2015-present
Luis Soto
Department of Radiation Oncology, *PhD Thesis Committee Member* 2015-present
Anh Diep

Faculty Search Committee

Department of Radiation Oncology, *Member* 2015-2018
Department of Obstetrics and Gynecology, *Member* 2015-2018

Residency Selection Committee

Department of Radiation Oncology, *Member* 2015-2018

Cancer Biology PhD Program Committees

Annual retreat organizing committee, *Chair* 2018
Annual retreat organizing committee, *co-Chair* 2017
Admissions committee, *Member* 2016-2017
Ovarian Cancer Focus Group Meeting, Organizer 2015-2017
Obstetrics and Gynecology Basic Research Seminar, co-Organizer 2017-present

Service to Professional Organizations

Membership

American Association for Cancer Research (AACR), *Member* 2015-present
Stanford Cancer Institute, *Member* 2015-present

Stanford Bio-X Program, *Member* 2015-present
Stanford Child Health Research Institute, *Member* 2015-present

Committee Service

American Society for Radiation Oncology (ASTRO), *Panel Member* 2016
Precision Medicine in Radiation Oncology: Personalizing Radiation Treatment. Bethesda, MD

Presentations

Invited Oral Presentations (National)

2017 Hypoxic signaling in Tumor-Mesothelial Niche Promotes Collagen Remodeling and Ovarian Cancer Metastasis. 15th International Tumor Microenvironment Workshop, Miami, FL
2016 Hypoxic signaling in ovarian cancer metastasis: Molecular mechanisms and targeted therapy. Third annual meeting of the international ovarian cancer consortium, Oklahoma City, OK
2011 Hypoxia inducible factor signaling in osteoblasts and the regulation of hematopoiesis. MGH Bone Research Workshop, Boston, MA
2010 The role of hypoxia signaling in the osteoblastic niche and the regulation of hematopoiesis, AACR, Washington DC
2005 ARNT is required for the development of VHL disease associated renal cysts in mice. ASN, Philadelphia, PA
2004 The role of hypoxia inducible factors in VHL disease associated tumorigenesis. ASN, St. Louis, MO

Invited Oral Presentations (International)

2017 Hypoxic signaling in the tumor-mesothelial niche. Keystone Symposia, Whistler, Canada
2016 Hypoxic signaling in tumor metastasis: molecular mechanisms and targeted therapy. The 3rd GI-CoRE Medical Science and Engineering Symposium, Hokkaido, Japan
2015 Hypoxic signaling in metastasis: Molecular mechanisms and targeted therapy. The Tumor Microenvironment Workshop, Vancouver, Canada
2008 HIF-2 regulates VHL associated vascular tumorigenesis and hepatic lipid metabolism in vivo. Keystone Symposia, Vancouver, Canada
2006 Hypoxic regulation of hepatic erythropoietin. International Conference on EPO, Lubeck, Germany

Poster Presentations

2017 Hypoxic signaling in the tumor-mesothelial niche promotes collagen remodeling and ovarian cancer metastasis. AACR: Ovarian Cancer Meeting, Pittsburgh, PA
2015 The receptor tyrosine kinase, AXL, is a therapeutic target driving the mesenchymal phenotype in ovarian cancer. AACR: Ovarian Cancer Meeting, Orlando, FL
2015 Direct regulation of GAS6/AXL signaling by HIF promotes renal metastasis through SRC and MET. Hypoxia Keystone Symposia, Dublin, Ireland
2014 Osteoblastic PHD signaling modulates the HSC niche. AACR Radiation Oncology Think Tank, Fort Myers, FL
2012 The HIF signaling pathway in osteoblasts directly modulates erythropoiesis through the production of EPO. Keystone Symposia, Banff, Canada
2011 Osteoblasts regulate erythropoiesis through HIF. Keystone Symposia, Big Sky, MO
2010 AXL is an essential factor and therapeutic target for metastatic ovarian cancer. Keystone Symposia, Keystone, CO

Teaching

2018 Lecturer CBIO 242: Hypoxia and Angiogenesis (Stanford University)

2017 Lecturer CBIO 242: Hypoxia and Angiogenesis (Stanford University)
2016 Lecturer CBIO 242: Hypoxia and Angiogenesis (Stanford University)
2016 Lecturer CBIO 280: Cancer Biology Journal Club (Stanford University)
2015 Instructor CBIO 280: Cancer Biology Journal Club (Stanford University)
2006 Teaching Assistant BIOM 555: Gene Expression (University of Pennsylvania)

Mentoring

Graduate Student

Joshua Eggold (NSF Fellowship)	2016-present

Postdoctoral Fellow

Suchitra Natarajan	2017-present
Jin Qian	2017-present
Yiren Xiao	2017-present
Hussein Shehade	2015-2017

Life Science Research Professional

Daniel Fregoso	2017
Katie Foreman	2016
Michaela Soriano	2015

Medical Resident/Fellow

Joseph Park	2015-2016
Karen Levy	2018-present